Research article

Cerebellar degeneration averts blindness-induced despaired behavior during spatial task in mice

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Abstract

Lurcher mutant mice of the C3H strain provide a model of both cerebellar and retinal degeneration. Therefore, they enable the study of the behavior of cerebellar mutants under disabled visual orientation conditions. We aimed to examine cerebellar Lurcher mutants and wild type mice with intact cerebellum with and without retinal degeneration employing the rotarod and Morris water maze tests. The positions of the hidden platform and the starting point in the water maze test were stable so as to enable the use of both idiothetic navigation and visual inputs. The Lurcher mice evinced approximately 90% shorter fall latencies on the rotarod than did the wild type mice. Retinal degeneration exerted no impact on motor performance. Only the wild type mice with normal retina were able to find the water maze platform efficiently. The wild type mice with retinal degeneration developed immobility (almost 25% of the time) as a sign of behavioral despair. The Lurchers maintained high swimming activity as a potential manifestation of stress-induced behavioral disinhibition and their spatial performance was related to motor skills and swim speed. We demonstrated that both motor deficit and pathological behavior have the potential to contribute to abnormal performance in spatial tasks. Thus, spatial disability in cerebellar mutants is most likely a complex consequence of multiple disturbances related to cerebellar dysfunction.

1. Introduction

In addition to its role in motor functions, the cerebellum has been proved to be involved in many non-motor functions such as behavioral and emotional control, attention, sensory input integration and spatial orientation [1–5]. Thus, damage to the cerebellum, including neurodegenerative diseases, causes a wide range of cognitive and affective impairments—cerebellar cognitive affective syndrome [1] which participates in the complex pathological phenotype of cerebellar patients.

Poorer spatial navigation in individuals suffering from cerebellar dysfunctions corresponds with recent findings that the cerebellum is involved in egocentric navigation and path integration [3,4]. The idea that the cerebellum contributes to this process has been supported by morphometric [6] and fMRI [7] studies on humans. Moreover, a study on mice with diminished cerebellar LTD showed that the cerebellum is crucial for proper hippocampal place cells firing in darkness [8], suggesting the cerebellum is a key structure for integration of proprioceptive, vestibular and motor signals during path integration [5].

Impairments in spatial navigation have also been reported in cerebellar degeneration mouse models, including Lurcher mutant mice [9], in which semi-dominant Grid2Lc mutation results in severe olivo-cerebellar degeneration [10]. Lurchers suffer from ataxia [11] and a wide range of cognitive and behavioral abnormalities [12–15] such as diminished behavioral control and deficits in terms of water maze navigation using external visual cues [16–18]. However, path integration and the importance of visual inputs for navigation in Lurchers has not previously been investigated. Furthermore, it is suspected that their performance in spatial tasks is influenced by behavioral disturbances [12,18], the importance of which, and the mechanisms employed to modify their spatial navigation strategy, are unclear. Thus, we can hypothesize that the conditions that enable both visual and idiothetic navigation and the more difficult conditions of disabled visual navigation exert differing impacts on the behavior of Lurcher and healthy mice. The subject of whether motor deficits limit the performance of cerebellar mutants in water mazes has also been discussed [16,18].

We aimed to examine the behavioral abnormalities of Lurcher mice during a spatial orientation task employing path integration. We also performed the rotarod test to assess the potential role of the motor

Abbreviations: MWM, Morris water maze; RD- Lc, Lurcher mice without retinal degeneration; RD + Lc, Lurcher mice with retinal degeneration; RD- WT, wild type mice without retinal degeneration; RD + WT, wild type mice with retinal degeneration

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disabilities of these mice and their relation to performance in the spatial task. To study these processes under conditions of disabled visual orientation, we used the C3H strain of Lurcher and wild type mice, where some of the subjects (homozygotes for the Pde6brd1 allele) suffered from retinal degeneration and, thus, a loss of vision [19].

2. Material and methods

2.1. Animals and design of the experiment

Heterozygous Lurcher mice and wild type mice (males and females) of the C3H strain aged 4.5–7.5 months were used. The mice were kept in a temperature- and humidity-controlled room with a 12/12 h light/dark cycle. Food and water were available ad libitum. All experiments reported here were conducted in full compliance with the European Union Guidelines for scientific experimentation on animals and with the permission of the Ethical Commission of the Faculty of Medicine in Pilsen. All efforts were made to minimize suffering.

The mice were examined using the Morris water maze (MWM) and rotarod tests, following which they were euthanized by an overdose of Thiopental. Hematoxylin-eosin-stained frozen slices of the mice eye-balls were histologically examined for the presence of retinal degeneration determined according to the absence of the outer nuclear layer (Supplementary Fig. 1). Cerebellar degeneration was identified according to obvious ataxia.

In order to be consistent with cerebellar mutant literature terminology, we use the term “wild type mouse” here for mice without cerebellar degeneration, regardless of the presence of retinal degeneration. Based on the presence of cerebellar and/or retinal degeneration, the following experimental groups were established: wild type mice without retinal degeneration (RD–WT, n = 17); Lurcher mice without retinal degeneration (RD–Lc, n = 22); wild type mice with retinal degeneration (RD+WT, n = 13); and Lurcher mice with retinal degeneration (RD+Lc, n = 8).

2.2. Morris water maze

The mice were examined in the MWM (round pool, 100 cm in diameter) with a circular escape platform (7.5 cm in diameter) hidden 0.5 cm below the water level for 17 days (D1-17). Four trials were performed in 20-min intervals each day with the same starting point (Supplementary Fig. 2). If the mice did not succeed in reaching the platform within 60 s, they were moved there manually. The mice were left on the platform for 30 s after each trial. A probe trial consisting of four 60-second trials without a platform was performed on D18. The mice were tracked employing the EthoVision XT system (Noldus Inc., Wageningen, the Netherlands).

For each day, we measured the following parameters: 1) latency (s) to locate the platform; 2) successful trials (%) indicating proportion of trials in which the mouse reached the platform with latency < 60 s; 3) non-moving (%) indicating proportion of time spent in non-moving state. Additional parameters are shown in the Supplementary Material. For the purpose of the analysis of the relation between the performance of the mice in the MWM and their motor abilities assessed as swim speed and performance on the rotarod, final performance and final swim speed were expressed as mean latency and swim speed respectively on the last two days (D16 and D17) of the MWM test. In the probe trial, we evaluated relative time in the target quadrant (% of time spent in the quadrant where the platform was located on D1-17).

2.3. Rotarod

To examine motor coordination, the rotarod (4 cm in diameter) accelerating from 0 to 60 rotations per minute over a period of 3 min was used (TSE Systems GmbH, Moos, Germany). Motor skills were expressed as mean fall latency from four subsequent measurements with 12 min intertrial intervals.

2.4. Statistical analysis

We used R software [20] for the statistical analysis. The analyses were extended by the permutation (4,999 randomizations) or re-sampling (10,000 simulations) approaches. Unequal variances in the residuals were stabilized using data transformation. Group comparisons were made employing permutational ANOVA. The significance of the differences between the expected 25 % and actual time in the quadrant during the probe trials was evaluated using the BCa bootstrap method [21] by boot [22] R package. Repeated measurements were analyzed via the permutational linear mixed-effects model (lme) with an AR1 autocorrelation structure using the nlme [23] and predictmeans [24] R packages. Post-hoc comparisons were performed using the (paired) permutation t-test followed by false discovery rate correction. In order to dissociate the partial effects of several predictors on the final performance, we employed linear models (LM) to obtain partial regression coefficients. Since four Lurcher mice (two RD- Lc and two RD+ Lc) jumped from the rotarod immediately, they were removed from the analysis. P < 0.05 was considered statistically significant.

Since the sex exerted no significant interaction with either the genotype or sight in any of the observed behavior (data not shown), this factor was not considered in the data analysis.

3. Results

3.1. Rotarod

For fall latencies in the rotarod test, significant effect of cerebellar degeneration (F(1,56) = 417.75; P < 0.0002) was found, while the effects of retinal degeneration and of interaction of both factors (cerebellar degeneration : retinal degeneration) were not statistically significant. The fall latencies of the Lurcher mice were reduced by approximately 90 % compared to the wild type mice, regardless of the presence of retinal degeneration (Fig. 1).

![Fig. 1. Mean fall latencies in the rotarod test in wild type (WT) and Lurcher (Lc) mice without retinal degeneration (RD-) and with retinal degeneration (RD+). Error bars represent 95 % confidence intervals. *** P < 0.001.](image-url)
3.2. Morris water maze

The significance of the effects of cerebellar degeneration, retinal degeneration, day of the MWM test and interaction of these factors on escape latency, successful trials %, and non-moving % are shown in Table 1. The distance moved, swim speed, direct swim % and thigmotaxis are shown in the Supplementary Material (Supplementary Table 1, Supplementary Fig. 3).

3.2.1. Cerebellar degeneration effect

The Lurcher mice without retinal degeneration attained more than 3-fold longer escape latencies and evinced almost half the frequency of successful trials than did the wild type mice with normal retinas (Fig. 2A, B). Nevertheless, both types of mice without retinal degeneration significantly improved in these parameters between D1 and D17 (Fig. 2A, B) and exhibited only minor differences regarding non-moving behavior (Fig. 2C). This parameter did not change significantly during the experiment.

3.2.2. Retinal degeneration effect

Retinal degeneration was associated in the wild type mice with 5-fold longer escape latencies, half the percentage of successful trials and a more than 5-fold higher percentage of the non-moving state (Fig. 2). Furthermore, the occurrence of the non-moving state increased significantly between D1 and D17 specifically in the blind wild type mice (Fig. 2C). Thus, the wild type mice with retinal degeneration formed the only experimental group that exhibited a high intensity of non-moving behavior and did not show any significant improvement during the MWM test (Fig. 2A, B).

In contrast, we found only a mild impact of retinal degeneration on performance in the MWM in Lurcher mice (Fig. 2). Surprisingly, Lurchers with retinal degeneration showed significant shortening of escape latencies, an increase of successful trials %, and low immobility % (Fig. 2A, B).

3.3. Morris water maze - Probe trial

Concerning the probe trial, only the wild type mice with normal retina spent significantly longer times in the target quadrant compared to the random distribution (Supplementary Fig. 4). The Lurcher mice with and without retinal degeneration spent approximately 25 % of the time in the target quadrant, indicating random distribution throughout the maze. In contrast, the wild type mice suffering from retinal degeneration remained in the vicinity of the starting point and spent significantly less time than expected in the target quadrant.

3.4. Partial effects

General linear models revealed that final latency in wild type mice was primarily related to the presence of retinal degeneration ($P < 0.001$), but not motor skills ($P = 0.8$) nor swim speed ($P = 0.1$). In contrast, final performance in Lurcher mutant mice was positively associated with swim speed ($P < 0.001$) and motor skills ($P = 0.01$), but was unrelated to retinal degeneration ($P = 0.75$) (Fig. 3; Supplementary Table 2).

4. Discussion

As mentioned previously [15,25], cerebellar degeneration in the Lurcher mice led to poor rotarod performance. Conversely, retinal degeneration...
We demonstrated here that the Lurchers also had a mild cerebellar motor function impairment. Vision did not help to compensate for degeneration exerted no impact on the test results for either the Lurcher or the wild type mice. Thus, vision did not help to compensate for cerebellar motor function impairment.

It was also demonstrated previously that the Lurcher mice have poor results in the MWM task based on alothetic navigation \[9,12,15,17,18\]. We demonstrated here that the Lurchers also had difficulties in the maze allowing for both alothetic and idiothetic navigation. The mild improvement in performance between the first and last days of testing seen in the Lurchers with both normal retinas and retinal degeneration suggested that some of the learning ability was preserved. Nevertheless, this should be attributed to procedural rather than to spatial learning due to the lack of preference for the target quadrant during the probe trial.

While the wild type mice with normal retina learned to find the platform easily, the Lurcher and wild type mice with retinal degeneration failed in this task, suggesting the inefficient use of non-visual idiothetic cues. Furthermore, the wild type mice with retinal degeneration developed behavioral despair manifested via immobility. Behavioral despair as a consequence of the difficulty of the water maze task has been highlighted previously \[26\]. This behavioral response might explain the absence of improvement in the blind wild type mice, i.e. their inactivity prevented any kind of learning.

The Lurcher mice showed marked problems navigating to the hidden platform, even those with normal retinas, which suggests that Lurchers have problems with the efficient use of distal visual cues. Although their navigation to the visually-marked target was inferior to that of the wild type mice \[9,16,17\], the visibility of the target noticeably improved their performance, thus demonstrating the partial ability to use a proximal visual cue \[17,18\].

The positive association of the results in the MWM with motor abilities suggests that motor disabilities could be one of the factors that determined the poor spatial task performance of the Lurchers. This is in contrast to a previous study by Lalonde and Thifault \[16\] who demonstrated the absence of correlation between MWM and coat hanger performances and considered spatial disabilities and motor deficits as independent factors in Lurcher mice. The rotarod test requires dynamic balance and good paw movement coordination that, together with swimming speed, may be more important for goal-directed movement in the MWM.

Both the blind Lurcher and wild type mice were unable to localize the platform even though the start and goal positions remained stable, thus enabling idiothetic navigation, which is in contrast to studies showing that rats and mice are able to use non-visual navigation \[8,27\]. Our experiment used permanently blind mice, while a study by Rochefort et al. \[8\] included training allowing visual navigation. We speculate that the task is too difficult for mice which have never had the chance to see the spatial arrangement of the maze and learn the path under less difficult conditions. Furthermore, any inter-study comparison is limited by inter-strain variability \[26\], and the performance of both the C3H wild type and Lurcher mice with normal retina was inferior to that of the B6CBA wild type and Lurcher mice respectively in the classic MWM test \[17\].

Despite the inability to navigate the MWM, the Lurcher mice did not develop the behavioral despair seen in the blind wild type mice. This observation complies with the behavior of the Lurchers in the forced swimming test \[18\]. Lalonde \[28\] reported fewer immobility responses and higher activity in Lurchers and suggested that these mice exhibit defective inhibitory mechanisms during swimming and locomotion. It has been suggested that Lurcher mice suffer from behavioral disinhibition potentiated or induced by anxiety and stress and manifested e.g. via reduced prepulse inhibition \[15\], higher exploration of the anxiogenic area, despite having a higher level of stress response \[13\] and abnormal behavior when confronted with predators \[14\]. It has been shown that the MWM task is a strong stressor for both wild type and Lurcher mice \[12\]. Nevertheless, the elevation of corticosterone following water-environment exposure was significantly higher in the Lurchers \[12\]. Although stress may promote certain compensations such as synaptic plasticity \[29\], the increased stress reaction to the water environment may induce behavioral disinhibition in Lurcher mice, thus reducing the chance to inhibit exploratory or escape behavior and, thereby, blocking the development of immobility responses and potentially interfering with an adequate platform searching...
strategy. Immobility might be considered an efficient strategy for avoiding exhaustion when escape is difficult. From this point of view, the Lurcher mice developed an inadequate response and failed to cope with the situation, which could be one of the mechanisms that participate in the development of anxiety in cerebellar patients [30].

5. Conclusion

The study confirmed the poor performance of Lurcher mice in the rotarod and MWM tests and identified an association between their motor and spatial deficits. While retinal degeneration exerted no impact on motor performance, it completely disabled orientation in the MWM, despite its arrangement allowing for idiothetic navigation. The Lurcher mice evinced a completely different behavioral response to the inability to escape from the MWM to the mice with intact cerebella. The lack of immobility responses may have been the result of stress-induced behavioral disinhibition in the Lurchers. Overall, we demonstrated that both the pathological behavioral response and motor deficit may contribute to abnormal spatial performance in cerebellar mutants. Thus, spatial disability is not just a simple consequence of the deteriorated specific contribution of the cerebellum to spatial navigation but a more complex product of multiple disturbances related directly or indirectly to cerebellar dysfunction.

Author contribution

Jan Cendelin: study design, investigation, result interpretation, manuscript writing, funding acquisition
Filip Tichanek: statistics, result interpretation, manuscript writing

Declaration of Competing Interest

None.

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Appendix A. Supplementary data

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References


